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Patentanmeldung Nr. Patent application No. Demande de brevet n°

99400239.2

PRIORITY DOCUMENT

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Der Präsident des Europäischen Patentamts;
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Page 2 de l'attestation

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Cell composition containing macro-phages, presenting anti-infectious and hematopoietic properties, and a process for preparing the same

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CELL COMPOSITION CONTAINING MACROPHAGES, PRESENTING ANTI-INFECTIOUS AND HEMATOPOIETIC PROPERTIES, AND A PROCESS FOR PREPARING THE SAME.

The invention relates to a cell composition containing macrophages, presenting anti-infectious and hematopoietic properties, and a process for preparing the same.

Peripheral hematopoietic apheresis instead of bone marrow puncture and purification are widely used to collect hematopoietic progenitor cells. These cells are used in allogeneic or autologous transplantations for the treatment of genetic diseases and mainly of neoplasic diseases to support high dose chemotherapy or radiotherapy.

Positive selection of the apheresis products for cells bearing the CD34 antigen ($CD34^+$ stem cells) and cryopreservation until use has become a recognized method.

However, it offers several drawbacks including cost (the process of selection of cells on antibody coated beads), limited amounts of progenitor cells recovered, delays in immune reconstitution (resulting in infectious complications in the post transplant period).

The number of progenitor cells present in blood can be increased by the pre-apheresis conditioning of the patient treated with GM-CSF and/or G-CSF colony stimulating factors and eventually with chemotherapy drug such as a cyclophosphamide.

However, cancer patients often relapse due to the presence of residual tumor cells resistant to the chemotherapy regimen (references of B. Klein).

An aim of the invention is to provide a new cell composition containing macrophages, presenting interesting properties in cancer immunotherapy and in stem cell transplantation.

Another aim of the present invention is to provide a new cell processing method allowing under improved standardized procedures the expansion of progenitor and stem cells from peripheral blood without costly purification of a defined cell population.

The aims of the invention are achieved by a cell composition containing macrophages, myeloïd cells and progenitor cells, with said progenitor cells being preferably present in a mean ratio of at least about 1 %, preferably about 0,1 to 20 %, with said myeloïd cells being preferably present in an amount of about 10 % to about 30 %, with said macrophages being preferably in an amount of about 10 to about 70 %, these percentages being expressed with respect to the total number of cells.

Macrophages, myeloïd cells and progenitor cells are defined as $CD14^+$ and $CD64^+$ cells (macrophages), $CD33^+$ cells (myeloid cells) and $CD34^+$ cells and/or GM-CFU (progenitor cells). GM-CFU are cells able to form colonies of granulocyte and macrophage in cytokine containing semi-solid culture medium after 14 days of culture.

According to an advantageous embodiment, the cell composition of the invention contains T lymphocytes, preferably in a ratio of about 10 to 60 %, expressed with respect to the total number of cells.

According to an advantageous embodiment of the invention, the progenitor cells contain from about 0,1 % to about 20 % of stem cells, expressed with respect to the total number of progenitor cells.

Stem cells are defined as expressing CD34 molecules and/or by their ability to form colonies in cytokine containing semi-solid culture medium.

According to an advantageous embodiment, the progenitor cells are generated from and possibly included in peripheral blood mononuclear cells, and in particular are chosen among :

myelo-erythroïd progenitor cells, myeloïd progenitor cells, lymphoïd progenitor cells or a mixture thereof.

The expression "included in" means that the progenitor cells are present in the cell composition.

The expression "generated from" means that the progenitor cells are differentiated from stem cells originally present in the cell composition.

In the cell composition of the invention, the macrophages, myeloïd cells and the lymphocytes if present, are included in/or generated from blood mononuclear cells.

The cell composition of the invention has gained a new combination of activities useful in cancer immunotherapy and in stem cell transplantation. These properties include :

1) purge by macrophages and cytotoxic T/NK cells of the tumor cells eventually present in the graft,

2) eradication of residual cancer disease in the patient by macrophages and/or antigen presenting cells (MAC-DCs dendritic cells) present in the autologous or in the allogeneic graft,

3) avoiding most infectious episodes after injection at the beginning of the aplasia period following therapy in the patient, thanks to the potent anti-viral, anti-bacterial and anti-parasite properties of macrophages and eventually of contaminating polynuclear cells and their precursors present in the product,

4) facilitating engraftment by the enhanced amount of stem cells, of hematopoietic cells, progenitors of myeloïd, erythroid and lymphoid as well as of cells at intermediate states of differentiation present in the graft,

5) decrease significantly of the aplasia period (correlated with patient's fever and infections) by markedly increasing the recovery rate of the different blood populations.

The invention also relates to a process for the preparation of a cell composition containing macrophages, myeloïd cells and progenitor cells, with said progenitor cells being preferably present in an amount of about 0,1 % to about 10 %, with said macrophages being preferably in an amount of about 10 to about 60 %, these percentages being expressed with respect to the total number of cells, comprising the step of mobilization of the progenitor cells in the blood of a patient, for instance by premedication of said patient with G-CSF and/or GM-CSF, or G-CSF and cyclophophosphamide, thus increasing the amount of progenitor cells in peripheral blood.

The term "mobilization" means stimulation of bone marrow cells to release increased amount of progenitor cells in the blood.

The process of the invention can comprise an additional step of coculture of the blood mononuclear cells and progenitors, after washing off the platelets, the granulocytes and erythrocytes, for about 4 to about 10 days, in a medium allowing differentiation of monocytes into macrophages and myeloïd progenitors into polynuclear cells.

According to an advantageous embodiment of the process, the coculture of the blood mononuclear cells and progenitors is carried out in the presence of cytokines or growth factors, for example : IL3, IL6, stem cell factor, EPO, thrombopoietin, GM-CSF, G-CSF, Flat-3 ligand, c-kit ligand or their agonists.

The process of the invention can also comprise an additional step of macrophage activation, at the end of the coculture, for instance by addition of γ-interferon or muramyl peptides.

The aim of activation macrophages is to gain more anti-infectious and anti-tumoral activity.

The process of the invention can comprise an additional step of concentration of the cells obtained at the end of the coculture, and resuspension in a vehicle suitable for administration to a patient.

The process of the invention can comprise after the resuspension of the coculture, a step of freezing part or the totality of the resuspension.

It is to be noted that cellular product obtained after *ex vivo* differentiation and expansion contains stem cells, progenitor cells, myeloïd cells, T lymphocytes and differentiated macrophages which are activated (for example by γ interferon) at the end of the process. The coculture for 3 to 12 days performed at 37°C in non adherent bags and defined medium (IMDM basis) allows increased recovery of $CD34^+$ cells and/or of intermediate hematopoietic progenitor cells. This means that normal hematopoietic progenitors are not only spared by activated macrophages, but are also stimulated to greater proliferation and differentiation.

The invention also relates to a cell composition such as obtained according to the process as defined above.

The invention also relates to a pharmaceutical composition containing, as active substance, a cellular composition as defined above.

The invention also relates to a method for the restoration of hematopoiesis in an aplasic patient and/or the protection of patients against infectious diseases or against residual tumors, comprising the use of a pharmaceutical composition as defined above.

The expression "restoration of hematopoiesis" means increasing the level of hematopoietic cells to reach a normal functionality similar to that of healthy individuals to achieve protection against infections (which can be measured by blood numeration and identification).

The invention also relates to a method for the diminution of the aplasia period in a patient, for instance from 11 days to 1 to 3 days, comprising the use of pharmaceutical composition as defined above.

The term "aplasia" is defined as a pathological low level of hematopoietic cells in blood.

The cell composition of the invention defined above is characterized by the fact that it is derived from and/or included in a peripheral blood mononuclear cell composition containing :

- from about 10 to about 50 % of monocytes,
- from about 10 to about 70 % of lymphocytes,
- from about 0,1 to about 20 % of progenitor cells,
- from about 1 to about 50 % of polynuclear cells,
- from about 0,1 to about 20 % of stem cells.

Example 1

A patient with multiple myeloma was treated with cyclophosphamide ($4g/m^2$) and a daily injection of G-CSF ($5\mu g/kg$). The peripheral blood CD34 count was monitored every day and apheresis was started when CD34 count was greater than $10/mm^3$. Mononuclear cells (less than 10 % polynuclear cells) were collected by

apheresis, washed twice in PBS, and cultured for 6 days in a MAK TM cell processor at a concentration of 5 x 10^6 cells/ml in 2 culture bags of 500 ml, at 37°C, 5 % CO_2. The culture medium contained 500 u/ml of GM-CSF. On day 6, IFN-gamma (250 U/ml) was added for one day. Before culture, and at day 1, 2, 5, 6, 7 of culture and after elutriation, a cell sample was harvested for determination of the cell count and of the percentages of CD34, CD14, CD33, CD64, HLA-DR, CD16, CD3 cells and of the concentration of granulocyte-macrophage colony forming units (GM-CFU). The cell membrane phenotype of the cells was determined by FACS analysis using FITC labeled murine monoclonal antibodies (Immunotech, Marseilles, France) or isotype control murine antibodies. The concentration of GM-CFU was determined using methylcellulose semi solid medium containing various hematopoietic cytokines purchased from Stemgen (Villejuif, France).

Progressive 3-fold increase of the numbers of CD34 cells and of GM-CFU has been observed. These data indicated that this culture system supported the growth of hematopoietic precursors.

Cells from apheresis (mobilized myeloma patients) could also be kept frozen. After thawing 5.7 x 10^9 cells containing 44 % $CD3^+$ cells (lymphocytes) and 42 % $CD14^+$ cells (monocytes) could be seeded and cultured in defined medium at 37°C.

Lymphocytes ($CD3^+$) and $CD14^+$ cells tended to decrease (see table 1). $CD64^+$ macrophages were low until activation by IFNγ at day 6, which increased markedly this population. This last population was mainly present after purification by elutriation at day 7.

When the subpopulation of $CD34^+$ stem cells was followed along the culture, it was observed an increase in the number and percentage of cells with $CD34^+$ phenotype. 7.10^7 $CD34^+$ cells were present at the beginning of culture, increasing to 21.10^7 after 6 days (Table 1).

The presence of cytokines released in the culture medium and of a large number of T lymphocytes (57 % in this experiment) appeared important for the proliferation of $CD34^+$ and progenitor cells. Macrophages were obtained reproducibly, with good viability after thawing. They could eventually be purified to 90 % by elutriation with and average of 5.10^9 macrophages differentiated from one apheresis (60 patients

analyzed). The total cell population recovered after culture is injected to patients to allow faster recovery of hematopoietic progenitors and decrease the period of aplasia.

Table 1

	Culture	J1	J2	J5	J6	Before Elutriation	After Elutriation
Volume (ml)	1000	1000	1000	1000	1000	1000	228
Cellular concentration (10^6/ml)	5	3.3	2.6	1.8	1.6	1.5	1.5
Total number of cells (10^9)	5	3.3	2.6	1.8	1.6	1.5	0.34
CD 3 (%)	57	33	27	15	23	19	1.3
CD 14 (%)	41	9.25	18	8.5	8	13	40
CD 16 (%)	34	11.25	4	.62	1	2	3.9
CD 64 (%)	28	nd	0.2	4.28	0.79	15	59
HLA-Dr (%)	60	nd	19	33.4	64	37	73
CD 33 (%)	nd	nd	nd	14	27	43	47
CD 34 (%)	1.41	nd	7.29	11	13	14	21
CFU-GM (/10^5)	116	nd	nd	nd	1000	nd	14
BFU (/10^5)	197	nd	nd	nd	2680	nd	nd

CLAIMS

1. Cell composition containing macrophages, presenting anti-infectious and hematopoietic properties.

2. Cell composition containing macrophages, myeloïd cells and progenitor cells, with said progenitor cells being preferably present in a ratio of at least about 0,1 %, preferably about 0,1 to 20 %, with said myeloïd cells being preferably present in an amount of about 10 % to about 30 %, with said macrophages being preferably in an amount of about 10 to about 60 %, these percentages being expressed with respect to the total number of cells.

3. Cell composition according to anyone of claims 1 or 2, containing T lymphocytes, preferably in a ratio of about 10 to 60 % expressed with respect to the total number of cells.

4. Cell composition according to anyone of claims 1 to 3, wherein the progenitor cells contain from about 0,1 % to about 20 % of $CD34^+$ stem cells, expressed with respect to the total number of progenitor cells.

5. Cell composition according to claim 4, wherein the progenitor cells are generated from and possibly included in peripheral blood mononuclear cells, and in particular are chosen among :

myelo-erythroïd progenitor cells, myeloïd progenitor cells, lymphoïd progenitor cells or a mixture thereof.

6. Cell composition according to anyone of claims 1 to 5, wherein the macrophages, myeloïd cells and the lymphocytes if present, are included in/or generated from blood mononuclear cells.

7. Process for the preparation of a cell composition containing macrophages, myeloïd cells and progenitor cells, with said progenitor cells being preferably present in an amount of about 0,1 % to about 20 %, with said macrophages being preferably in an amount of about 10 to about 60 %, these percentages being expressed with respect to the total number of cells, comprising the step of mobilization the progenitor cells in the blood of a patient, for instance by premedication of said patient with G-CSF and/or GM-CSF, or G-CSF and cyclophophosphamide, thus increasing the amount of progenitor cells in peripheral blood.

8. Process according to claim 7, comprising an additional step of coculture of the blood mononuclear cells and progenitors, after washing off the platelets, the granulocytes and erythrocytes, for about 4 to about 10 days, in a medium allowing differentiation of monocytes into macrophages and myeloïd progenitors into polynuclear cells.

9. Process according to claim 8, wherein the coculture is carried out in the presence of cytokines or growth factors, for example : IL3, IL6 stem cell factor, EPO, trhombopoitein, GM-CSF, G-CSF, Flat-3 ligand, C-kit ligand or their agonists.

10. Process according to anyone of claims 8 or 9, comprising an additional step of macrophage activation, at the end of the coculture, for instance by addition of γ-interferon or muramyl peptides.

11. Process according to anyone of claims 6 to 10, comprising an additional step of concentration of the cells obtained at the end of the coculture, and resuspension in a vehicle suitable for administration to a patient.

12. Process according to claim 11, comprising, after the resuspension of the coculture, a step of freezing part or the totality of the resuspension.

13. Cell composition such as obtained according to the process of anyone of claims 7 to 12.

14. Pharmaceutical composition containing, as active substance, a cellular composition according to anyone of claims 1 to 6 or 13.

15. Cell composition according to anyone of claims 1 to 6 or 13, charaterized by the fact that it is derived from and/or included in a peripheral blood mononuclear cell composition containing :

- from about 10 to about 50 % of monocytes,
- from about 10 to about 70 % of lymphocytes,
- from about 0,1 to about 20 % of progenitor cells,
- from about 1 to about 50 % of polynuclear cells,
- from about 0,1 to about 20 % of stem cells.

16. Use of a cell composition according to anyone of claims 1 to 6 or 13, for the preparation of drugs, for the restoration of hematopoiesis in an aplasic patient and/or the protection of patients against infectious diseases or against residual tumors.

ABSTRACT

CELL COMPOSITION CONTAINING MACROPHAGES, PRESENTING ANTI-INFECTIOUS AND HEMATOPOIETIC PROPERTIES, AND A PROCESS FOR PREPARING THE SAME.

The invention relates to a cell composition containing macrophages, presenting anti-infectious and hematopoietic properties.

More particularly, the invention relates to a cell composition containing macrophages, myeloïd cells and progenitors ; said cell compositions are useful for the restoration of hematopoiesis in an aplasic patient and/or the protection of patients against infectious diseases or against residual tumors.

(*no figure*)